

# Human serum antibodies reacting with endotoxin from *Neisseria gonorrhoeae*

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The chemical composition and serological properties of endotoxin prepared from gonococci by extraction with aqueous ether have been described in previous reports (Maeland, 1969b, 1969c). Aqueous ether endotoxin contains one antigenic determinant of carbohydrate nature (determinant *a*) and another antigenic determinant of protein nature (determinant *b*) (Maeland, 1968, 1969a). A serological test system based on indirect haemagglutination technique has been described for each determinant (Maeland, 1969c). The *b* determinant has been shown to possess serological group-reactive properties and is also present in meningococci (Maeland, 1969d). The *a* determinant comprises several antigenic factors called *a* factors (Maeland, 1969d). One *a* factor was shared by all of 27 strains of gonococci examined, whereas other *a* factors occurred in different combinations among strains of gonococci.

In recent years several reports on human antibodies to gonococcal antigens have appeared but few describe antibodies to antigens which have been isolated and characterized. A study of the reactivity of human sera with each of the determinants *a* and *b* of endotoxin from gonococci was therefore undertaken. This paper describes the results obtained with sixty human sera.

## Material and methods

### HUMAN SERA

Serum from 30 patients (patient sera) and from thirty healthy blood donors (normal sera) were examined. The patient sera were obtained from individuals with a bacteriologically verified diagnosis of gonorrhoea who were treated at the Outpatients Clinic for Venereal Diseases, The University Hospital, Bergen. Twenty of the patients presented with gonorrhoea for the first time and ten had previously been treated for this disease. Six patients suffered from complications associated with gonorrhoea: arthritis (3), salpingitis (2), epididymitis (1). All sera were heat-inactivated at 56°C. for 30 min.

### PREPARATION OF ANTIGEN

Aqueous ether endotoxin was prepared from *N. gonorrhoeae* strain V (kindly provided by Dr. Alice Reyn,

Copenhagen) as described previously (Maeland, 1968, 1969a). In order to obtain preparations which sensitized erythrocytes with each of the determinants *a* and *b*, the endotoxin was further treated with periodate or pronase as described earlier (Maeland, 1969c).

### SENSITIZATION OF SHEEP ERYTHROCYTES

This was performed by adding a solution of the antigen preparation (0.5 mg./ml.) to an equal volume of a 1 per cent. suspension of sheep erythrocytes. The mixture was incubated at 37°C. for 30 min., washed, and finally prepared as a 0.5 per cent. suspension in phosphate-buffered saline pH 7.2.

### ANTI-HUMAN GLOBULIN SERUM

Antiserum to human serum globulins was prepared by immunization of rabbits with the animal's own erythrocytes agglutinated by pooled, inactivated, normal human serum (Tönder and Milgrom, 1966). The antiserum contained antibodies to the IgA, IgG, and IgM classes of human globulins as revealed by immunoelectrophoretic analysis. The haemagglutination titre of the antiserum was estimated by titration against human O R<sub>1</sub>R<sub>2</sub> erythrocytes sensitized with excess of incomplete human anti-D antibodies.

### INDIRECT HAEMAGGLUTINATION (HA) TEST

Serum was absorbed with unsensitized sheep erythrocytes. Two-fold dilutions of the absorbed serum were prepared in phosphate-buffered saline in volumes of 0.1 ml., and equal volumes of sensitized erythrocytes were then added. An antigen control and a control for complete absorption of the normal agglutinins to sheep erythrocytes were included. The racks were left at 4°C. for 20 hrs and the agglutination was read by the pattern technique.

### ANTIGLOBULIN (AG) TEST

Two-fold dilutions of the human serum were incubated at 4°C. for 20 hrs with erythrocytes sensitized with determinant *a* or *b*. The erythrocytes in each tube were then washed twice with buffered saline and 0.2 ml. diluted rabbit anti-human globulin serum containing 128 agglutinating units of antibodies were added. Incubation and reading of the agglutination were performed as for the HA test.

## GEL FILTRATION OF SERUM

Sera were subjected to gel filtration on a column (2.5 × 50 cm.) of Sephadex G-200. Volumes of 2.5 ml. of patient serum or normal serum were applied. The normal sera had been concentrated approximately three times by dialysis against polyethyleneglycol. The column was run with 0.05 M tris-buffer pH 8, containing 0.14 M NaCl and 0.02 per cent. sodium azide. The effluent was collected in fractions of 5 ml. and the transmission at 254 mμ was determined.

## REDUCTION BY 2-MERCAPTOETHANOL (ME)

Equal volumes of 0.2 ME and human serum diluted 1 : 2 or undiluted fractions from the Sephadex column, were mixed and incubated at 37°C. for 30 min. The mixtures were tested for serological activity without further treatment.

## Results

*Titres of untreated and ME-treated sera in the HA test*

Nearly two-thirds of the normal sera reacted in the HA test with determinants *a* and *b* (Table I). Of the patient sera all but three gave agglutination with these determinants. Most sera gave titres ranging from 8 to 32, although sera from some patients gave titres which were two to eight times higher than those obtained with any of the normal sera. Several sera displayed activity with only one of the determinants, either *a* or *b*.

TABLE I *Grouping of sera according to titres in the indirect HA test of human serum antibodies to determinants a and b of endotoxin from N. gonorrhoeae*  
*Tests performed with untreated sera and ME-treated sera*

Serum	Antigen	Number of sera with titre:								Total number of sera tested
		< 8	8	16	32	64	128	256	512	
Normal Untreated	Determinant <i>a</i>	10	10	8	2					30
	Determinant <i>b</i>	11	8	7	2	2				30
ME-treated	Determinant <i>a</i>	30								30
	Determinant <i>b</i>	30								30
Patient Untreated	Determinant <i>a</i>	3	2	8	6	9	2			30
	Determinant <i>b</i>	3	6	6	7	4	3		1	30
ME-treated	Determinant <i>a</i>	26	1		1	2				30
	Determinant <i>b</i>	24	1		3	1	1			30

TABLE II *Grouping of sera according to titres in the AG test of human serum antibodies to determinants a and b of endotoxin from N. gonorrhoeae*  
*Tests performed with mercaptoethanol-treated sera*

Serum	Antigen	Number of sera with titre:										Total number of sera tested	
		< 8	8	16	32	64	128	256	512	1,024	2,048		4,096
Normal	Determinant <i>a</i>	9	3	4	10	3	1						30
	Determinant <i>b</i>	5	2	1	6	9	7						30
Patient	Determinant <i>a</i>				5	1	7	4	4	2	6	1	30
	Determinant <i>b</i>			4	2	6	6	7	3			2	30

The activity of all normal sera in the HA test was destroyed by treatment of the sera with ME. On the other hand, serum from four patients reacted with determinant *a* and serum from six patients reacted with determinant *b* after treatment with ME. None of these treated sera combined with both of the antigenic determinants. Thus serum from ten of the patients contained ME-resistant antibodies reacting in the HA test with the endotoxin complex. Of six patients with diagnosed complications five had ME-resistant serum antibodies reacting in the HA test with determinant *a* or *b*.

*Titres of ME-treated sera in the AG test*

Table II shows the results obtained in the AG test with all ME-treated sera. Most normal sera reacted in this test with determinants *a* and *b* and with titres ranging from 8 to 128. Sera which were active in the HA test before treatment with ME usually displayed activity in the AG test after such treatment but a few sera were active in only one of the tests.

All ME-treated sera from patients reacted in the AG test with both determinants and with titres ranging from 16 to 4096. With determinant *a*, seventeen sera gave titres > 256, as did twelve sera with determinant *b*. The titres obtained tended to parallel each other, in that most sera which gave titres > 256 with one determinant also gave titres of

the same order of magnitude with the other. A total of eighteen sera from patients gave titres in the AG test  $\geq 256$  with both determinants or with one of them. All sera from patients with complications associated with gonorrhoea gave titres  $\geq 256$  with both determinants. Those sera which were active in the HA test after treatment with ME gave titres which were 4 to 64 times higher in the AG test than in the HA test.

#### *Gel filtration of serum*

This was performed with four sera, two from patients and two from healthy individuals. Untreated fractions were examined in the HA test and ME-treated fractions in the HA and AG tests. Normal serum and patient serum which, after reduction with ME, were inactive in the HA test gave essentially the same results after gel filtration. With both sera only fractions which corresponded to the first absorption maximum were active in the HA test and this activity was destroyed by treatment of the fractions with ME (Figure). The activity in the AG test of ME-treated serum was found to reside in the fractions which corresponded to the second absorption maximum.

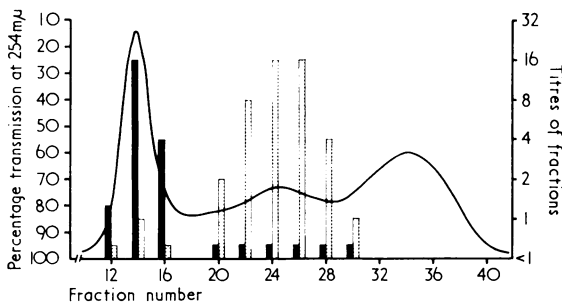


FIGURE Gel filtration on Sephadex G-200 of serum from a patient with gonorrhoea. Antibody activity of fractions to determinant *a*

Solid: Titres of untreated fractions in the indirect haemagglutination test  
Shaded: Titres of ME-treated fractions in the anti-globulin test.

Patient serum which contained ME-resistant antibodies active in the HA test were subjected to gel filtration. The fractions corresponding to the second absorption maximum, in addition to being active in the AG test, also reacted in the HA test. The activities of the fractions were not affected by treatment with ME. The fractions of the first absorption maximum contained ME sensitive antibodies active in the HA test.

#### **Discussion**

Serum from patients with gonorrhoea and from healthy blood donors have been examined for antibodies to determinants *a* and *b* of endotoxin from gonococci using indirect haemagglutination techniques. Similar results were obtained with both determinants in spite of the fact that determinant *a* is of carbohydrate and determinant *b* of protein nature (Maeland, 1968, 1969a).

The majority of normal sera contained antibodies active in the indirect HA test but in low titres. This activity was probably due to so-called natural antibodies. The presence in human serum of natural antibodies to antigens in gonococci has been reported previously (Cohen, 1967; Cohen and Norins, 1966; Maeland, 1966). Nearly all sera from patients reacted in the HA test. The activity in this test of all the reacting normal sera and of most sera from patients was due to IgM antibodies, as indicated by the results of ME-reduction and gel filtration of sera. The comparatively small differences in titre observed in the HA test between normal sera and patient sera indicate that during infection with gonococci the stimulus to produce IgM antibodies reactive with the endotoxin complex is very weak. The present study thus corroborates the findings of Cohen (1967). By the indirect fluorescent antibody method, he was unable to demonstrate clear-cut differences between the reactivity of natural and immune human IgM antibodies to gonococcal antigens.

More obvious differences between the two groups of sera emerged from the results obtained in the HA test with ME-treated sera. Ten sera from patients gave agglutination with determinant *a* or *b*, while none of the normal sera reacted. That the activity of ME-treated sera in the HA test was due to antibodies of the IgG class was further supported by results of gel filtration experiments.

The activity of ME-treated sera in the anti-globulin test (AG test) could also be attributed to the IgG class of globulins which in most sera behaved as incomplete IgG antibodies. This observation bears a certain resemblance to the results reported by Cohen, Norins, and Julian (1967). By means of the indirect fluorescent antibody procedure they demonstrated IgG antibodies in normal sera reacting with antigens in gonococci but found that these antibodies reacted weakly or not at all in the bacterial agglutination test. According to Cohen (1967), serum from patients with gonorrhoea did not contain an increased amount of IgG antibodies to heat-stable gonococcal antigens. These antigens probably include determinants *a* and *b* (Maeland, 1968). The results reported by Cohen are therefore at

variance with those of the present study, since serum from many patients gave higher titres in the AG test as compared with the titres of normal sera.

The higher titres found in the AG test with many of the ME-treated patient sera as compared to normal sera support the view that, in the former group, part of or all of the incomplete antibodies are immunoglobulins formed in response to infection with gonococci. There is, however, the possibility that the activity in the AG test of ME-treated normal sera is due to a non-specific attachment of serum globulins to the endotoxin complex and not to an antigen-antibody reaction.

It is possible that many more of the patient sera contained IgG antibodies able to react in the HA test but present in concentrations too low to be detected with serum diluted 1 : 8. The results of pilot experiments (unpublished) with more concentrated sera lends support to this view.

In the present study antigens from only one strain of gonococci (strain V) were used. The *b* determinant is serologically group-reactive, while determinant *a*, as shown with rabbit antisera, comprises several antigenic factors present in various combinations among strains of gonococci (Maeland, 1969d). It is therefore reasonable to suppose that with serum from patients infected with gonococci having an *a* determinant identical to that of strain V, the antibody titres found express the true amount of antibody present. In other cases, when the *a* determinant of the infecting strain is not identical to that of strain V, the titres found probably do not express the true amount of antibodies present to determinant *a*. Further experiments are needed to solve these problems.

### Summary

Serum from thirty patients with gonorrhoea and from thirty healthy blood donors was examined for antibodies to determinants *a* and *b* of endotoxin from gonococci using indirect haemagglutination techniques. Nearly all of the sera from patients and two-thirds of the normal sera contained antibodies of the IgM class to these determinants. With most patient sera the titres of the IgM antibodies appeared comparable to the titres of normal sera. One-third of the patient sera also contained antibodies, presumably of IgG class, which reacted in the indirect haemaggluti-

nation test. Antibodies of this type could not be detected in serum from healthy donors.

All sera from patients and the majority of those from healthy individuals contained antibodies which could be detected by means of an anti-human globulin serum. Many of the patient sera gave elevated titres of these antibodies. It is suggested that this finding was due to a rise in incomplete IgG antibodies in patients with gonorrhoea.

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### Anticorps du sérum humain réagissant avec l'endotoxine du *Neisseria gonorrhoeae*

#### SOMMAIRE

En utilisant des techniques d'hémagglutination indirecte, on a étudié les sérums de 30 malades atteints de gonococcie et de 30 donneurs de sang sains pour la recherche d'anticorps vis-à-vis des déterminants *a* et *b* de l'endotoxine gonococcique. Presque tous les sérums des sujets malades et les deux tiers des sérums normaux contiennent des anticorps IgM par rapport à ces déterminants. Pour la plupart des sérums de malades, les titres des anticorps IgM se montrèrent comparables aux titres des sérums normaux. Un sur trois des sérums de malades contenait aussi des anticorps, sans doute du groupe IgG, qui réagissaient dans l'épreuve d'hémagglutination indirecte. Des anticorps de ce type ne purent pas être trouvés dans le sérum des donneurs sains.

Tous les sérums des malades et de la majorité des sujets sains contenaient des anticorps qui pouvaient être trouvés en utilisant une globuline sérique anti-humaine. Beaucoup des sérums provenant de malades présentèrent des titres élevés de ces anticorps. On pense que cette constatation est due à une augmentation des anticorps IgG incomplets chez les malades atteints de gonococcie.